SLEEP RHYTHMS IN THE GOLDEN HAMSTER: EFFECTS OF VARIOUS COMPLETE AND SKELETON PHOTOPERIODS

By

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The author wishes to express his graditude to the hamsters who gave their lives to make this research possible. Special thanks are also due Wilse B. Webb who served as the author's chairman and who, more importantly, provided the author with the opportunity to pursue his research interests to an unusual extent and with a rare degree of freedom.

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By

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Chairman: Wilse B. Webb
Major Department: Psychology

Photoperiod length is the environment's most noise-free indicator of the season of the year. The relative duration of light and darkness each day is commonly used by organisms to produce seasonally appropriate changes in their physiological characteristics and behavioral activity. In addition, light has immediate effects on these variables in direct response to its presence. In the case of rodent sleep short light-dark cycles (1 hour light: 1 hour dark, for example) have been shown to produce an enhancement of total sleep time during the lights-on periods with paradoxical sleep being pushed from the light periods during which it normally occurs to the dark periods.

These findings suggest that photoperiod duration may have effects on rodent sleep variables which are not taken into account in typical rodent sleep studies. In addition, in the wild rodents sleep in burrows and are consequently exposed to light only near dawn and dusk. To date, sleep research in rodents has only employed photoperiods which run continuously from dawn to dusk without regard to the ecological history of the species.

To assess the effects of photoperiod duration on rodent sleep 18 hamsters were prepared for standard electrographic sleep recording and assigned to a photoperiod of either 6, 12, or 18 hours duration. Each photoperiod group was further divided into a complete photoperiod group and a skeleton photoperiod group. The complete photoperiod groups were exposed to light each day for the total duration of their photoperiods. The skeleton photoperiod groups were exposed to light only at the beginning and the end of their assigned photoperiods, thus mimicking the natural condition. After a minimum of 45 days on the experimental photoperiods sleep was assessed. A second experiment was then conducted with 12 animals run on the 6 hour photoperiod paradigm, but with only 14-21 days of adaptation time to the experimental photoperiods.

A true photoperiodic effect on sleep amounts was not found. A 2% to 4% reduction of sleep time did occur under both the long and short adaptation 6 hour photoperiod conditions apparently because of the very short duration of the daytime phase when a nocturnal species would normally sleep. The magnitude of the sleep rhythm and its placement in time did not differ substantially between complete and skeleton photoperiod conditions, but it was "pushed around" by photoperiod duration such that its maximum tended to occur near the middle of the photoperiod. There was no significant dissociation of slow wave sleep and paradoxical sleep rhythms. Use of complete photoperiods appears to be valid in rodent sleep research.

INTRODUCTION

This study is intended to define the role of the photoperiod in determining the rodent sleep response. The first problem in assessing the role of the photoperiod in sleep is to determine whether or not sleep is an endogenous circadian rhythm. This has been established by several studies (Dube and Kilduff, in preparation; Mitler et al., 1977; Webb and Dube, in press) which demonstrate that mammalian sleep is an endogenous circadian rhythm with the properties such rhythms normally possess. In rodents this has been demonstrated by maintaining both rats (Dube and Kilduff, in preparation) and mice (Mitler et al., 1977) in constant light and constant dark and observing that the sleep rhythm remains intact with a free running period of approximately 24 hours.

The photoperiod can affect a circadian rhythm in two ways; it can determine the placement of a rhythmic variable within the 24 hour day and it can affect the mean level of a rhythmic variable. The process of placement control is called entrainment and has been well studied in rodents, particularly in the case of light as the entraining agent or "zeitgeber." When a reference point of a rhythm is chosen (such as activity onset) its placement under various lighting conditions can be used as an indicator of the effect of that particular lighting condition on rhythm placement. A typical example is provided by the effect of a 10 min. light pulse applied at various phases of the activity rhythm of a hamster otherwise maintained in constant darkness.

A plot of the time of occurrence of the light pulse versus the magnitude of the phase shift of the activity onset produced by it leads to a phase response or light sensitivity curve of the sort shown in Figure 1. Here a daily cycle is defined relative to the animal with the time of his activity onset taken as time 0, his inactive phase taken as his subjective day and his active phase taken as his subjective night. As this plot shows, if a 10 min. period of white light is presented 2 hours after the animal has begun his daily wheel running, on subsequent days he will begin his running 60 min. later than he would have if constant darkness had been maintained. Toward the end of his subjective night a light pulse causes the animal to begin his activity earlier than he otherwise would have. Phase shifts are achieved by a 1 or 2 day lengthening or shortening of the activity rhythm until the new phase relationship exists.

These phase response curve results suggest how entrainment is accomplished in nature. If the animal begins his activity too early he will be subjected to the light of dusk which will produce a later onset the following day. If, on the other hand, he begins his activity too late in the night and it runs into the light of dawn, then he will begin his activity earlier the next day. Thus, in nature entrainment of rhythms like hamster activity is usually achieved at dawn and dusk. The animal is unresponsive to light during his subjective day when he expects it and at night, barring unusual circumstances, he is not exposed to it. This is further reflected in the fact that light intensity only affects the magnitude of the phase shift at values between 0 lux and 10 lux (very dim) which is a very short intensity range only occurring at dawn and dusk (Dunning, 1973).

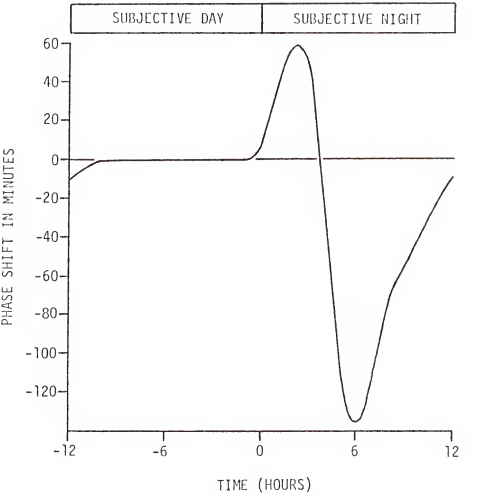


Figure 1. Phase response curve for phase shifting by light of hamster wheel running activity onset. Baseline represents time relative to activity onset (DeCoursey, 1964).

As noted above the photoperiod can also affect the mean level of a rhythmic variable or, for that matter, the magnitude of a non-rhythmic variable. This is often achieved by the process referred to as photoperiodism. Since photoperiod length is the environment's most noise-free indicator of the season of the year, the relative duration of light and darkness each day is commonly used to produce seasonally appropriate changes in the physiological characteristics and behavioral activity of animals. In organisms as diverse as insects, birds and mammals photoperiod length is the initial determinant in the causal chain leading to reproductive activity. In birds, critical photoperiod lengths trigger moulting and migration, while in insects they may halt development in a "diapause" strategically timed in advance of unfavorable conditions (Hillman, 1973; Pittendrigh, 1974). In the golden hamster short photoperiods lead to gonadal regression and cessation of sexual activity while photoperiods greater than 12.5 hours produce the opposite effects (Elliott, Stetson and Menaker, 1972; Reiter, 1973). In addition to the all-or-none effects currently known to be controlled by photoperiod length, the fact that the temperature tolerance (Pittendrigh, 1961) and growth rate (Saunders, 1972) of insects are photoperiodically modulated suggests a broader spectrum of phenomena may be involved.

Considerable evidence exists that photoperiodic effects in mammals are the result of a timing process which measures the duration of either the light or dark period with the use of an endogenous circadian rhythm (Pittendrigh and Minis, 1964; Pittendrigh, 1974). This mechanism does not require continuous exposure to light, but only brief exposure at dawn and dusk. The circadian system then, in

effect, times the interval between the two. Photoperiods mimicking this exposure to light have been termed "skeleton" photoperiods and have been found effective in entraining circadian rhythms (Pittendrigh and Minis, 1964). Since a good deal of this work has employed hamsters (Mesocricetus auratus) as subjects, they were chosen as subjects for this study in order to assess the effect of photoperiod length on the magnitude of sleep parameters.

Irrespective of photoperiodism is the fact that in the wild hamsters, and rodents commonly used in sleep research, sleep in burrows. Thus, they are exposed to light only near dawn and dusk, in essence the skeleton photoperiod condition. To date, sleep research in rodents has employed complete photoperiods without regard for the ecological history of the species. This is not only an unnatural condition for burrowing species such as Rattus norvegicus and Mesocricetus auratus, but one which may distort the sleep response. Borbely et al. (1975) have shown that rats on an LD 1:1 (light 1 hour: dark 1 hour) cycle experience an enhancement of total sleep time during the lights-on periods and further that paradoxical sleep tends to occur in the dark immediately following lights-on, with the magnitude of the effect greatest during the animal's subjective day. Similar results have been reported by Johnson, Adler and Sawyer (1970) and Fishman and Roffwarg (1972). In addition, pinealectomy in rats has recently been shown (Mouret et al., 1974) to eliminate the rhythm of paradoxical sleep and pineal compounds are known to be highly responsive to the presence of light (Axelrod, 1974). The presence of light during the subjective day, therefore, may be distorting the sleep pattern of burrowing nocturnal rodents. Because of these effects skeleton photoperiods as well as

complete photoperiods were studied here in order to separate the effects of light as a stimulus directly affecting sleep from the effects of day length per se. This comparison of complete and skeleton photoperiods of equal length thus tests the validity of using complete photoperiods in rodent sleep research.

A further consideration leading to the present study derives from a theory of sleep function proposed by Webb (1971, 1974, 1975). This theory postulates adaptive non-responding as the main function of sleep. He presents arguments which attempt to relate the environmental conditions under which the organism must exist (food availability, predator status) to the amount and placement of sleep. For example, at certain times of the day food gathering may be ineffective or dangerous to a particular species due to lack of availability and/or predator pressure. Continued activity under such circumstances is wasteful of energy and dangerous. At such times the non-responding of sleep is adaptive and enhances chances for survival. Thus, he suggests, a species by species comparison can be made correlating each species ecological niche, and need to non-respond, with its sleep pattern.

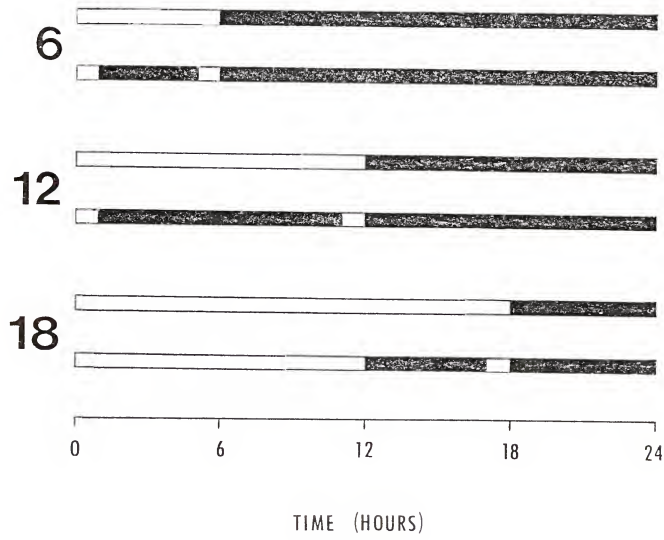
In examining this theory, however, one must consider the variability of a given species' environment. While food may be plentiful in the summer it may be scarce in the winter and so on. Thus, if the function of sleep is the sophisticated non-responding that the adaptive non-responding theory proposes, one would expect the sleep system to meet these changing requirements. Since photoperiod length is such an important factor in triggering responses to seasonal changes, one would expect changes in photoperiod length to produce appropriate modifications of sleep. Thus, one purpose of the present study was to test the adaptive non-responding theory of sleep.

EXPERIMENT 1

The design of this experiment is presented in Figure 2. Three photoperiod durations were studied 6, 12, and 18 hours. In addition, each photoperiod was investigated as both a complete and a skeleton photoperiod. In each case the portion of the light-dark cycle intended to represent daytime is referred to as the photoperiod. When considered from the animal's point of view the term subjective day is used. The terms lights-on and lights-off refer to the actual condition of the white light sources.

With the use of symmetric skeleton photoperiods, for example lights-on from 800 to 900 hours and 1300 to 1400 hours, there are two possible interpretations of day length. In nature photoperiod length changes a few minutes each day and animals gradually adjust to each new photoperiod. In the laboratory, where the direction of change in length cannot be inferred from a sudden switch in the light-dark cycle, animals generally choose the shorter of the two alternatives. Thus, in order to simulate a long photoperiod length a long period of lights-on must occur to make the desired day length the shorter of the two possible interpretations. The asymmetric skeleton photoperiod of 18 hours shown in Figure 2 was designed with this in mind.

Figure 2. Schematic representation of the six photoperiods investigated. Open sections of the bars represent lights-on and dark sections lights-off. Numbers at the left of each pair give photoperiod durations in hours.


6
12
18
0
6
12
18
24
TIME (HOURS)

Method

Subjects

Eighteen male golden hamsters (Mesocricetus auratus) of the LVG:Lak outbred strain were used. They were obtained at 60 days of age from Charles River Hamster Colony, Lakeview, New Jersey. Their body weights averaged 113.6 grams at the beginning of the study.

Upon arrival in the laboratory the animals were placed in individual cages (standard Wahmann Co.) measuring 24.5 cm. long, 20.0 cm. wide and 18.0 cm. high, with 0.8 cm. wire mesh tops and 1.28 cm. wire mesh floors and fronts. Food (Purina Laboratory Chow) was placed on the floor of the cages and water was available ad lib from a bottle mounted on the front of each cage. All animals were housed in the same room and maintained on an LD 12:12 cycle. Room temperature was maintained at 21±1°C. These conditions were maintained for approximately 14 days to allow the animals to adapt to the laboratory.

Surgery

To prepare the animals for standard electrographic sleep recording, surgery was performed after the initial 14 day adaptation period. All subjects were prepared under Nembutal (sodium pentobarbital) anesthesia, 100mg/Kg, administered intraperitoneally, with supplementary doses administered when needed.

Under clean, but not sterile, conditions electrodes were implanted using a Kopf Instrument Co. stereotaxic instrument. Four stainless steel screw electrodes were located over the cortex in the areas previously found by this laboratory to be the most suitable for recording slow waves and theta rhythms. All electrodes were connected via insulated wire to female Amphenol mini-connector pins which were imbedded in a plastic block. The plug and wires were then cemented to the skull. Following surgery the animals were returned to their home cages and allowed to recover. Tetracycline was provided in their water for 3 days.

Pre-recording Procedure

Following 1 week of recovery from surgery, the animals were randomly divided into six squads of three each. Each squad was placed in a light-tight ventilated isolation box measuring 40 cm. wide, 66 cm. long, and 64 cm. high. All isolation boxes were maintained in the same quiet experimental room. Animals were kept in the cages they were placed in upon arrival in the laboratory so that each isolation box contained three individual cages housing one animal each. Each isolation box was maintained on one of the experimental photoperiods shown in Figure 2. White light was provided by a General Electric 25 watt soft-white incandescent bulb mounted 31 cm. above the cages which provided an intensity of 280 lux at the cage floors. All photoperiod onsets occurred at 700 hours EST as did the LD 12:12 photoperiod onset during the adaptation and surgery phases. Continuous access to food and water and a temperature of approximately 21°C were

maintained throughout. Routine maintenance was performed at irregular intervals and only at times when the house light was on.

Recording Procedure

Following 7 to 10 weeks of exposure to the experimental photoperiods a 24 hour recording period was undertaken for each squad. For purposes of recording each animal was placed in a cage exactly like his home cage except for a plexiglass top and a wire mesh back where the water bottle was placed. During this procedure each animal was examined for gonadal condition as a verification of the effects of the photoperiods. The animals were then placed in a recording chamber exactly like the isolation boxes except for the presence of mercury commutators mounted above the cages. Each animal was then connected to a Grass Model IV EEG machine by a four conductor shielded cable plugged into the commutator above his cage. The recording chamber was then placed in front of a one-way window facing an adjacent room containing the recording equipment. Within the recording box illumination was provided by a white light of the same sort present in the isolation boxes (280 lux at cage floors) and by a red light (General Electric red ceramic coated 25 watt incandescent bulb) which was used to view the animals during the lights-off phases. The experimental photoperiod of each squad was maintained throughout the recording procedures.

Following 2 days of habituation to the leads a continuous 24 hour ECoG recording was made with three channels derived from each animal at a paper speed of 5mm/sec. Visual observation was also

carried out during the recording sessions with the animals' behavior written on the ECoG chart as a cross check on the ECoG sleep score. Following recording the animals were sacrificed (Nembutal overdose) and examined for electrode position and condition. All were found to be satisfactory.

Data Analysis

The ECoG records were visually scored on an epoch by epoch basis as either waking, slow wave sleep (SWS) or paradoxical sleep (PS), according to the criteria of Van Twyver (1969). No criterion was used for minimum epoch length; any clearly identifiable sleep stage was scored as such.

The following variables were then analyzed for each group.

1. SWS time. The total amount of time spent in slow wave sleep.
2. PS time. The total amount of time spent in paradoxical sleep.
3. TST (total sleep time). The sum of SWS and PS times.
4. PS/TST (%). The percentage of total sleep time spent in paradoxical sleep.
5. Sleep Cycle Length. The length of time from the end of one PS episode to the end of the next one. Only periods uninterrupted by waking were counted.
6. Sleep Epoch Length. The duration of a period of SWS, with or without PS, both preceded and followed by more than one minute of waking.
7. SWS Epoch Length. The duration of each individual episode of SWS.
8. PS Epoch Length. The duration of each individual episode of PS.

9. Corrected Day-Night Sleep Ratio. The mean TST/hr for the photoperiod phase divided by the mean TST/hr of the night phase.

Each of these variables was analyzed by a 3x2 (photoperiod x completeness) factorial ANOVA (see appendix). To test for differences between the photoperiod phase and the night phase of variables 4-8, they were computed separately for each phase and then analyzed with paired t-tests blocking on animals.

Placement Analysis

To determine the placement of sleep two measures were used, the amplitude (A) and the time of occurrence of the maximum (θ) of each 24 hour time series. A and θ were chosen (1) because they give an indication of the degree of the rhythmicity of each variable and (2) because they provide the most meaningful and applicable measure of sleep distribution when photoperiod length is varied.

These parameters were estimated by making a least squares fit of the sine curve to the data with a separate estimate obtained for SWS and PS for each experimental condition. The computations were performed as follows. With f = 3 values of the dependent variate y at each of the k = 24 one hour intervals within the cycle, the curve defining the expected response Y at each t has the equation $Y = a + A(\cos(ct - \theta))$ where $a = \bar{y}$ is the mean of the y's for all $N = fk = 72$ observations. The coefficient A is one-half the range in Y from the maximum to the minimum, designated here as the amplitude. The constant $c = 2\pi/k$ converts time measured in hours to angular measure in radians. The statistic θ is the phase angle in radians, or the time at which

the response Y is a maximum, as measured in this case from the onset of each photoperiod.

Since the cosine and sine are orthogonal and additive transformations of time, the constants in the above equation were determined from the regression coefficients b and c in the alternative form $Y = a + b[\cos(ct)] + c[\sin(ct)]$. The amplitude A was computed using the formula $A = (b^2 + c^2)\exp(1/2)$. The phase angle θ was estimated from the ratio of these same two coefficients $\tan\theta = c/b$ with θ then converted from radians to hours using $t_{max} = k\theta/2\pi = 24\theta/6.2832$.

The coefficients b and c were computed in a manner similar to that for the coefficients of orthogonal polynomials for successive powers of x. Thus, for k = 24 equally spaced intervals in a complete cycle, the cosines and sines corresponding to the successive intervals of time, $t = 0, 1, 2, \ldots, (k-1)$, form an orthogonal set of two independent variates within a negligible rounding error. With $u = \cos(ct)$, $v = \sin(ct)$, the above regression equation may be written as $Y = a + bu + cv$ where $\sum u = \sum v = \sum(uv) = 0$. The regression coefficients b and c were thus computed as $b = [\sum(uT_t)]/[(1/2)fk]$ and $c = [\sum(vT_t)]/[(1/2)fk]$ where T_t is the total of the f = 3 observations at each time t (Bliss, 1958, 1970).

Results

Sleep Amounts

Tables 1-3 present a summary of sleep measures for each photoperiod. TST was significantly different ($p < .05$) between photoperiods, while completeness had no effect. This result was due to the 6 hour photoperiods (Figures 3 and 4) which had about 4% less sleep time than the 12 and 18 hour groups. This effect was slightly greater for PS than SWS but not enough for significant differences in PS/TST percentage between groups (Figure 5).

While PS/TST percentage did not differ significantly between photoperiods or completeness, it was significantly different between day and night ($t = 10.87$, $df = 17$, $p < .001$). Day values averaged 26.24% while night values averaged 17.03%.

Epoch Lengths

Neither mean sleep cycle length, mean sleep epoch length, mean SWS epoch length nor mean PS epoch length differed between photoperiod or completeness conditions.

Sleep epoch length did differ significantly between day and night ($t = 7.26$, $df = 17$, $p < .001$). Day values averaged 16.20 min. while night values averaged 21.75 min.

TABLE 1

Summary of Values for the Six Hour Photoperiod

Complete Photoperiod	Skeleton Photoperiod
Mean % of Total Sleep Time	
58.59	56.41
Range = 57.43 - 60.56	Range = 52.08 - 60.56
Mean % of Paradoxical Sleep/Total Sleep Time	
22.23	21.26
Range = 21.75 - 22.82	Range = 20.13 - 22.33
Mean Sleep Cycle Length (Mins)	
8.30	7.37
Range = 7.40 - 9.90	Range = 6.80 - 7.90
Mean Sleep Epoch Length (Mins)	
18.20	18.83
Range = 16.30 - 20.80	Range = 16.30 - 20.80
Mean Slow Wave Sleep Epoch Length (Mins)	
5.63	5.57
Range = 4.10 - 8.20	Range = 4.90 - 6.20
Mean Paradoxical Sleep Epoch Length (Mins)	
2.40	2.30
Range = 1.90 - 2.70	Range = 2.10 - 2.70
Mean Corrected Day-Night Sleep Ratio	
1.45	1.55
Range = 1.36 - 1.51	Range = 1.45 - 1.66

TABLE 2

Summary of Values for the Twelve Hour Photoperiod

	Complete Photoperiod	Skeleton Photoperiod
Mean % of Total Sleep Time		
	59.58	62.36
	Range = 58.19 - 60.63	Range = 61.67 - 65.00
Mean % of Paradoxical Sleep/Total Sleep Time		
	22.91	22.14
	Range = 21.60 - 23.99	Range = 21.17 - 23.18
Mean Sleep Cycle Length (Mins)		
	7.73	7.53
	Range = 7.00 - 8.10	Range = 7.00 - 8.10
Mean Sleep Epoch Length (Mins)		
	17.63	17.00
	Range = 16.70 - 19.40	Range = 16.70 - 17.50
Mean Slow Wave Sleep Epoch Length (Mins)		
	5.37	5.07
	Range = 5.10 - 5.80	Range = 4.90 - 5.20
Mean Paradoxical Sleep Epoch Length (Mins)		
	2.50	2.47
	Range = 2.30 - 2.80	Range = 2.20 - 2.60
Mean Corrected Day-Night Sleep Ratio		
	1.62	1.45
	Range = 1.43 - 1.81	Range = 1.38 - 1.53

TABLE 3

Summary of Values for the Eighteen Hour Photoperiod

	Complete Photoperiod	Skeleton Photoperiod
Mean % of Total Sleep Time		
	63.80	59.79
	Range = 60.83 - 66.67	Range = 58.40 - 61.94
Mean % of Paradoxical Sleep/Total Sleep Time		
	22.38	23.49
	Range = 21.25 - 23.17	Range = 22.20 - 24.61
Mean Sleep Cycle Length (Mins)		
	8.30	8.10
	Range = 7.60 - 9.20	Range = 7.00 - 9.20
Mean Sleep Epoch Length (Mins)		
	19.43	18.13
	Range = 16.70 - 20.80	Range = 17.50 - 19.40
Mean Slow Wave Sleep Epoch Length (Mins)		
	5.87	6.00
	Range = 4.70 - 7.10	Range = 4.40 - 7.00
Mean Paradoxical Sleep Epoch Length (Mins)		
	2.50	2.27
	Range = 2.40 - 2.70	Range = 2.00 - 2.40
Mean Corrected Day-Night Sleep Ratio		
	1.70	2.15
	Range = 1.20 - 2.20	Range = 1.65 - 2.68

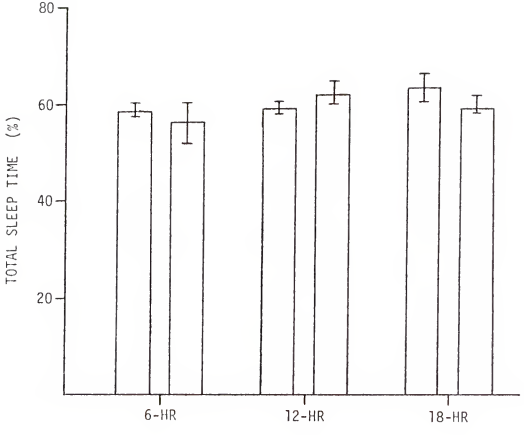


Figure 3. Percentage of TST for each experimental group. The complete photoperiod value is represented by the left bar of each pair and the skeleton photoperiod value is represented by the right bar. The line at the top of each bar indicates the range.

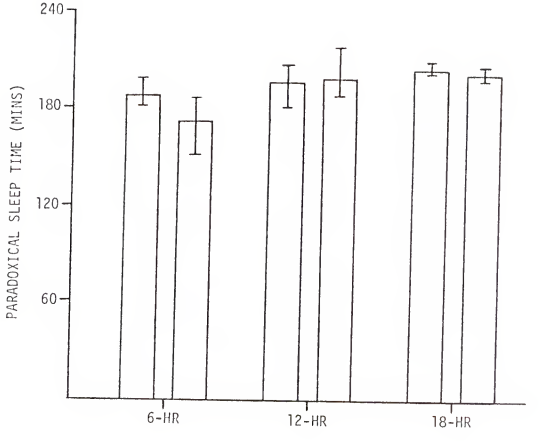


Figure 4. Amount of PS for each experimental group. The complete photoperiod value is represented by the left bar of each pair and the skeleton photoperiod value is represented by the right bar. The line at the top of each bar indicates the range.

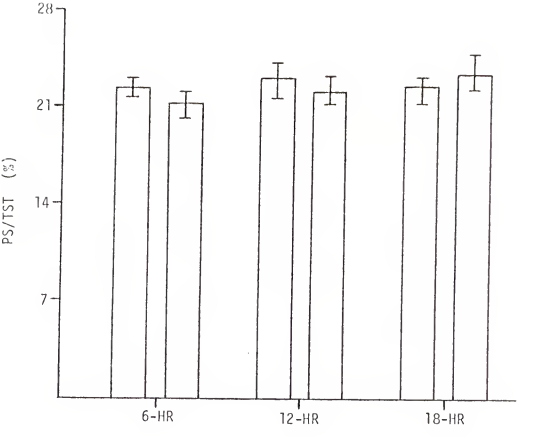


Figure 5. Percentage of TST spent in PS for each experimental group. The complete photoperiod value is represented by the left bar of each pair and the skeleton photoperiod value is represented by the right bar. The line at the top of each bar indicates the range.

TABLE 4

Day and Night Values of PS/TST (%) for each Experimental Group

		6 Hour	12 Hour	18 Hour
Complete	Day	29.7	26.4	23.6
	Night	18.7	17.1	16.4
Skeleton	Day	25.8	27.4	24.5
	Night	18.9	14.4	16.7

Placement Analysis

The mean corrected day-night sleep ratios did not differ significantly between photoperiod or completeness conditions. The values for the 18 hour photoperiods did tend to be higher (Figure 6); however, their range was also larger and overlapped the other groups considerably.

Figures 7-9 show the mean SWS and PS distributions for each experimental group. Table 4 gives the values of the phase angles and amplitudes for each group. These results indicate no significant differences between completeness conditions. However, photoperiod durations have a clear cut effect on sleep distribution. As photoperiod duration increases the time of the maximum moves to the right tending to assume a position slightly to the left of the center of the photoperiod. There is no significant dissociation of SWS and PS rhythms in the placement of their maximums. The amplitudes vary slightly with photoperiod duration with the 6 hour photoperiods having slightly less amplitude than the others. The 18 hour skeleton photoperiod has a more sharply defined SWS rhythm than the other groups with an estimated peak to peak range of 22.1 min.

TABLE 5

Values of Phase Angle and Amplitude for each Experimental Condition

		Phase Angle (Hours)		Amplitude (Mins.)	
		SWS	PS	SWS	PS
6-Hour	Complete	1.85	1.98	4.81	4.15
	Skeleton	2.65	0.93	5.44	3.24
12-Hour	Complete	4.07	5.39	6.70	5.13
	Skeleton	7.92	6.72	6.52	5.94
18-Hour	Complete	6.21	6.43	6.32	3.82
	Skeleton	6.91	7.02	11.05	5.16

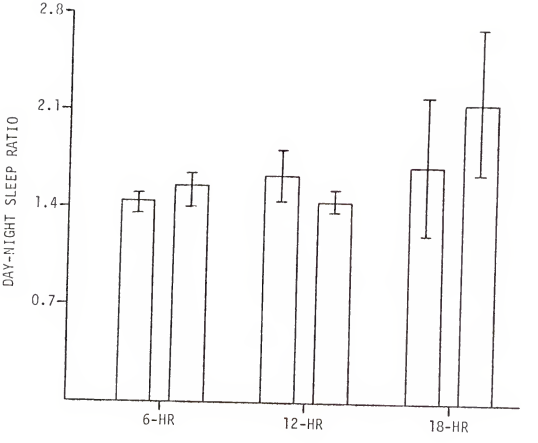
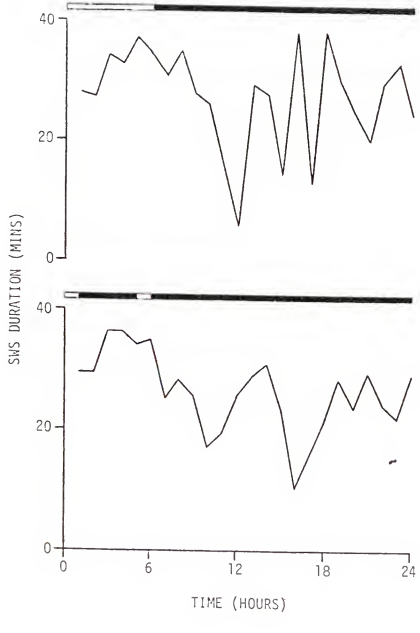


Figure 6. Corrected day-night sleep ratio for each experimental group. The complete photoperiod value is represented by the left bar of each pair and the skeleton photoperiod value is represented by the right bar. The line at the top of each bar indicates the range.

Figure 7. Mean hourly distribution of SWS and PS for 6 hour photoperiods. Bars above each graph designate lights-on (open sections) and lights-off (dark sections).


SWS DURATION (MINS)
40
20
0
40
20
0
0
6
12
18
24
TIME (HOURS)

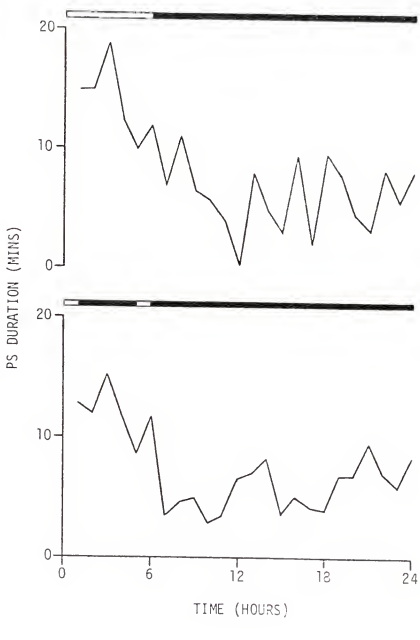
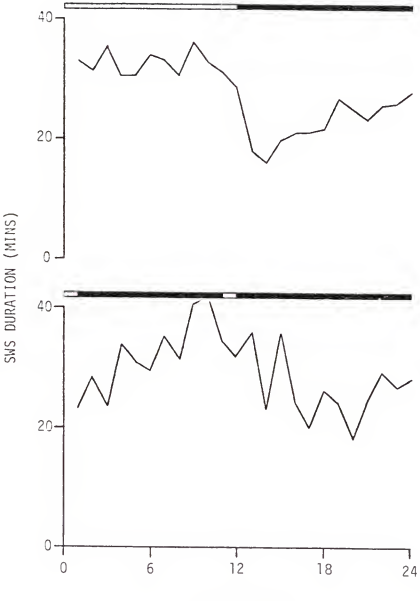

PS DURATION (MINS)
20
10
0
20
10
0
0
6
12
18
24
TIME (HOURS)

Figure 8. Mean hourly distribution of SWS and PS for 12 hour photoperiods. Bars above each graph designate lights-on (open sections) and lights-off (dark sections).


SWS DURATION (MINS)
40
20
0
40
20
0
0
6
12
18
24
TIME (HOURS)

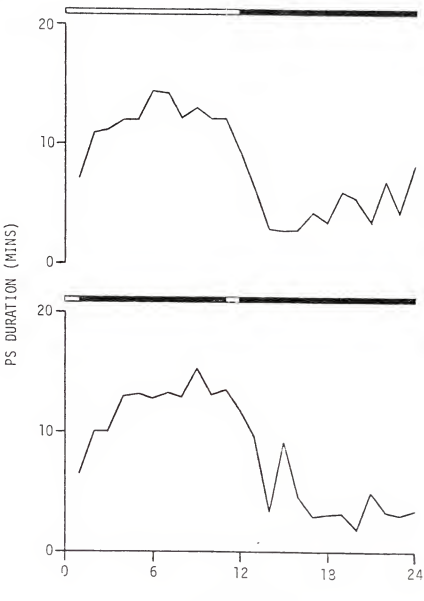
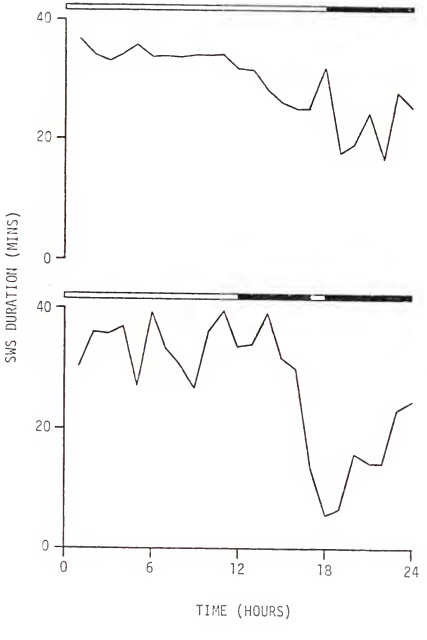
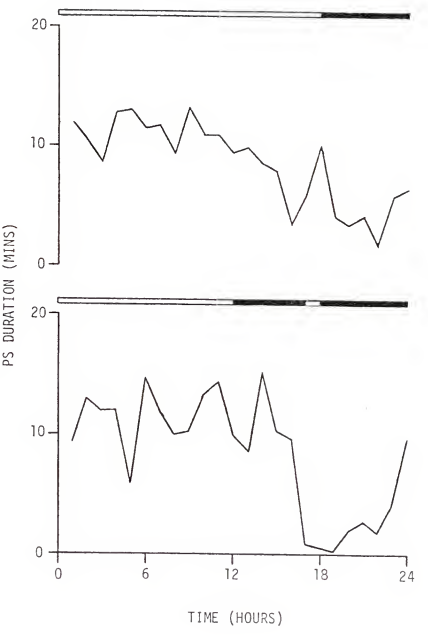

PS DURATION (MINS)
20
10
0
20
10
0
0
6
12
18
24
TIME (HOURS)

Figure 9. Mean hourly distribution of SWS and PS for 18 hour photoperiods. Bars above each graph designate lights-on (open sections) and lights-off (dark sections).


SWS DURATION (MINS)
40
20
0
40
20
0
0
6
12
18
24
TIME (HOURS)


PS DURATION (MINS)
20
10
0
20
10
0
0
6
12
18
24
TIME (HOURS)

EXPERIMENT 2

Because of the irregularity of the sleep pattern for the 6 hour photoperiod groups, and in order to demonstrate more firmly the ability of the photoperiod to control sleep placement, another group of animals was run on the 6 hour photoperiods. In this case, however, the adaptation time on the experimental photoperiods was shortened to 2 weeks. This short adaptation time was chosen to demonstrate that the small effect on sleep amounts in the 6 hour groups was not likely to be a photoperiodic phenomenon and to demonstrate the ability of the photoperiod to gain control of the sleep distribution in a relatively short period of time.

Method

The general procedures followed in this experiment were the same as in Experiment 1. Twelve male hamsters were obtained at 90 days of age from the same source. Their body weights averaged 123.6 grams.

Upon arrival in the laboratory they were handled in an identical manner to the animals in Experiment 1. Following approximately 14 days of adaptation to an LD 12:12 cycle they were surgically prepared for electrographic sleep recording. The animals were then placed in the isolation boxes with six animals maintained on a complete 6 hour

photoperiod and six animals maintained on a skeleton 6 hour photoperiod (1 hour L: 4 hour D: 1 hour L: 18 hour D).

Following 2-3 weeks on the experimental photoperiods sleep recording procedures were instituted. The same recording procedures and data analyses were performed as in Experiment 1.

Results

Table 5 presents a summary of sleep measures for each condition, while Figure 10 shows the SWS and PS distributions obtained. Again completeness had no effect on sleep amounts. Furthermore, the values obtained here are similar to those obtained in Experiment 1 indicating the shorter adaptation time allowed in this case was still sufficient for the photoperiod condition to exert its influence on sleep variables.

PS/TST (%) was again significantly different between day and night ($t = 10.85$, $df = 11$, $p < .01$). Day values averaged 28.42% while night values averaged 19.62%. Sleep epoch length was also significantly different between day and night ($t = 7.26$, $df = 11$, $p < .01$). Day values averaged 16.98 min. while night values averaged 22.33 min. No other variables achieved statistically significant differences.

TABLE 6

Summary of Values for the Six Hour Photoperiods of Experiment 2

Complete Photoperiod	Skeleton Photoperiod
Mean % of Total Sleep Time	
57.59	57.30
Range = 52.03 - 60.55	Range = 52.01 - 61.80
Mean % of Paradoxical Sleep/ Total Sleep Time	
22.05	23.03
Range = 20.13 - 23.59	Range = 22.12 - 24.71
Mean Sleep Cycle Length (Mins)	
8.07	7.97
Range = 6.80 - 9.90	Range = 7.40 - 9.20
Mean Sleep Epoch Length (Mins)	
19.58	18.62
Range = 16.30 - 20.80	Range = 16.50 - 20.80
Mean Slow Wave Sleep Epoch Length (Mins)	
6.18	5.42
Range = 4.90 - 8.20	Range = 4.10 - 7.10
Mean Paradoxical Sleep Epoch Length (Mins)	
2.25	2.48
Range = 1.90 - 2.70	Range = 2.10 - 2.70
Mean Corrected Day-Night Sleep Ratio	
1.47	1.52
Range = 1.36 - 1.66	Range = 1.43 - 1.72

Figure 10. Mean hourly distribution of SWS and PS for 6 hour photoperiods of Experiment 2. Bars above each graph designate lights-on (open sections) and lights-off (dark sections).

SWS DURATION (MINS)
40
20
0
0
6
12
18
24
TIME (HOURS)
PS DURATION (MINS)
20
10
0
0
6
12
18
24
TIME (HOURS)

DISCUSSION

The results of this study are in accord with those of numerous other studies (for example: Dube, 1976; Webb and Friedmann, 1971) which have found the amount of sleep an organism obtains to be extremely resistant to modification. While a difference in sleep amounts did just achieve statistical significance in this study, the magnitude of the difference was quite small and clearly not the result of a photoperiodic phenomenon. A photoperiodic response mechanism could not be expected to exist to produce such a small response which, with a magnitude of 2% to 4%, produces no real modification of the animal's sleep pattern. Furthermore, as demonstrated by Experiment 2, the change in sleep amounts occurs over a very short period of time. Given the time course of the hamster gonadal photoperiodic response, for example, it might be expected that any photoperiodically induced change in sleep amounts might occur on a more gradual basis. It appears, therefore, that the slightly reduced sleep amount of the 6 hour photoperiod groups was the result of these nocturnal animals exhibiting their natural tendency to obtain the majority of their sleep during the daytime, but being too severely restricted by the abnormally short day length to do so.

The results of the 6 hour groups are similar to those obtained by restricting food access to a brief period during the daytime (Dube, 1976). In the restricted feeding case significant waking time

is introduced into the daytime causing an abnormally low daytime sleep total. These animals then compensate by greatly elevating their nighttime sleep totals even though they have a natural tendency (biological rhythm) toward wakefulness at this time. The compensation is large enough to prevent any statistically significant loss of sleep even though their day-night ratios are less than one. The patterns assumed here by the 6 hour photoperiod animals (Figures 7 and 10) are very similar to those of the restricted feeders; they have an abnormally low amount of sleep during the day (due to its short duration) and they then show an elevated nighttime level which compensates for the daytime deficit. In this case the compensation is inadequate, but only minimally so.

The difference found in daytime and nighttime PS/TST values (Table 4) is, in effect, a rhythm of PS/TST paralleling the TST rhythm. Thus, this tendency for PS to concentrate more in the daytime than SWS is also reflected in the amplitudes of the PS and SWS rhythms given in Table 5. Even though the absolute amounts of the two sleep stages are much different the amplitudes of their rhythms are relatively close. The existence of this difference among the skeleton photoperiod groups as well as the complete photoperiod groups indicates that this is not simply the result of the sort of stimulus effects of light on sleep mentioned earlier, but rather the result of a true PS rhythm with a higher relative amplitude than the SWS rhythm.

This difference in day-night PS/TST values does not appear to be dependent on the length of SWS epochs preceding PS epochs either. In this study there was no statistically significant difference in

the length of daytime and nighttime SWS epochs. Furthermore, it was not uncommon to see a rather short SWS epoch precede a fairly lengthy PS episode and vice versa. This indicates that the PS rhythm is actually independent of the SWS rhythm except that the PS rhythm cannot express itself if SWS does not occur.

This is in agreement with considerable human data which shows PS to have a rhythm which can be dissociated from the SWS rhythm (Webb and Dube, in press). In the human case PS tends to occur during the early morning hours whether the subject is allowed to go to bed at his normal time or is forced to stay awake for the first half of the night. Further, the rhythm of human PS is maintained during any naps the subjects take with morning naps high in PS and afternoon naps low in PS.

The significance of the PS/TST rhythmicity in rats is unknown; however, the peak of these PS/TST rhythms always seems to occur 180° out of phase with the peak of gross bodily activity, especially activity like wheel running as opposed to maintenance activities like grooming or eating. Quantitative relationships between activity amounts and PS amounts, however, have not been established.

The significance of the difference between day and night values of sleep epoch length is difficult to determine. Because of the arbitrary nature of the amount of waking chosen as marking the beginning and end of an epoch, it is possible that other values might affect the result. One possibility, however, is suggested by the PS/TST results discussed above. Since the PS/TST values are lower

at night while daytime and nighttime average PS epoch lengths are not significantly different, the relative number of PS episodes to SWS episodes is lower during the night than during the day. It is possible that the animals have a greater chance of experiencing a waking episode long enough to be recorded as such after a PS episode than after an SWS episode. This might be the result of the loss of muscle tone which occurs during PS. At the start of a PS episode the animals tend to fall over into postures considerably different from those they assume at the beginning of sleep. This often results in considerable postural adjustment and thus significant waking time at the end of PS episodes. While postural adjustments often occur during SWS episodes they are usually minor and result in only brief ECoG desynchronization. Thus, the greater need to make postural adjustments at the end of PS episodes could be the reason for the sleep epoch values, as defined, to be longer at night than they are during the day. It is interesting to note that this day-night difference in sleep epoch length is in agreement with results obtained in the cotton rat (Sigmodon hispidus) which is also a member of the family Cricetidae (Kilduff and Dube, 1976).

The results of the sleep placement analysis clearly indicate the ability of the photoperiod to control the temporal aspects of rodent sleep. Most fundamental in this regard is the ability demonstrated by the light-dark cycle to entrain the sleep rhythm, particularly in the case of skeleton photoperiods which have not previously been studied with regard to the sleep rhythm. This firmly puts rodent sleep into the category of rhythms which are strongly entrained by light.

In this case, however, the role of light is more than that of a simple entraining agent; it is also effecting the waveform of the rhythm. The distribution of sleep across the 24 hours is being strongly controlled by photoperiod duration. If sleep were a rhythm entrained by light, but nothing more, the waveform of sleep would be the same for each photoperiod group. For example, if the sleep rhythm merely locked onto some reference point, such as photoperiod onset, there would be no difference in the time of the maximum between groups. The fact that the maximum of the sleep rhythm tends toward the center of the photoperiod indicates that day length is influencing the placement of sleep.

Most important is the lack of significant differences between complete and skeleton photoperiods. This indicates that it is day length and not the direct effects of light per se which is responsible for the temporal positioning of sleep. If light were acting in a direct stimulus-response fashion the skeleton photoperiods would be expected to produce no effect at all or at the most an effect during the two light pulses. The fact that the sleep distribution peaks near the middle of the skeleton photoperiods just as it does near the middle of the complete photoperiods indicates some other type of mechanism is at work. It also firmly establishes the validity of using complete photoperiods in sleep research employing burrowing nocturnal rodents.

The final problem to consider here is the significance of these results to the adaptive non-responding theory of sleep. Since varying photoperiod lengths did not profoundly affect sleep amounts this study fails to support the theory; however, it does not contradict it. As a hibernator the hamster possesses a mechanism

which enables him to make adaptive behavioral modifications in response to seasonal changes in his environment. Thus, sleep could still serve the function proposed in the theory during the times of the year when the animal is not hibernating, when his sleep pattern presumably mates him well with his environment. Therefore, it is difficult to draw any conclusions about the function of sleep from this study.

To summarize, there is no profound difference in the amount of sleep between photoperiod or completeness conditions. The difference previously found to exist in daytime and nighttime values of PS/TST was found here under all conditions indicating that it is not an effect induced by the presence of light during the day. The length of sleep epochs is greater during the night than during the day. Most importantly the validity of using complete photoperiods in sleep research employing burrowing nocturnal rodents has been established.

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APPENDIX

TABLE 7

Summary of Analysis of Variance of Total Sleep Time

Source	SS	df	MS	F	p
Photoperiod	12926.778	2	6463.389	4.656	.05
Completeness	1200.500	1	1200.500	0.865	
Photoperiod x Completeness	7660.333	2	3830.167	2.759	
Within Cell	16660.000	12	1388.333		

TABLE 8

Summary of Analysis of Variance of Paradoxical Sleep Time

Source	SS	df	MS	F	p
Photoperiod	1769.444	2	884.722	5.690	.05
Completeness	122.722	1	122.722	0.789	
Photoperiod x Completeness	224.778	2	112.389	0.723	
Within Cell	1866.000	12	155.500		

TABLE 9

Summary of Analysis of Variance of Paradoxical Sleep/Total Sleep Time

Source	SS	df	MS	F	p
Photoperiod	4.348	2	2.174	2.017	
Completeness	0.201	1	0.201	0.186	
Photoperiod x Completeness	3.937	2	1.969	1.827	
Within Cell	12.939	12	1.078		

TABLE 10

Summary of Analysis of Variance of Corrected Day-Night Sleep Ratios

Source	SS	df	MS	F	p
Photoperiod	0.668	2	0.334	3.408	
Completeness	0.076	1	0.076	0.776	
Photoperiod x Completeness	0.290	2	0.145	1.480	
Within Cell	1.177	12	0.098		

TABLE 11

Summary of Analysis of Variance of Sleep Cycle Length

Source	SS	df	MS	F	p
Photoperiod	0.991	2	0.496	0.618	
Completeness	0.889	1	0.889	1.107	
Photoperiod x Completeness	0.538	2	0.269	0.335	
Within Cell	9.640	12	0.803		

TABLE 12

Summary of Analysis of Variance of Sleep Epoch Length

Source	SS	df	MS	F	p
Photoperiod	7.324	2	3.662	1.094	
Completeness	0.845	1	0.845	0.253	
Photoperiod x Completeness	2.893	2	1.447	0.432	
Within Cell	40.147	12	3.346		

TABLE 13

Summary of Analysis of Variance of Slow Wave Sleep Epoch Length

Source	SS	df	MS	F	p
Photoperiod	1.543	2	0.772	0.515	
Completeness	0.027	1	0.027	0.018	
Photoperiod x Completeness	0.142	2	0.071	0.047	
Within Cell	17.993	12	1.499		

TABLE 14

Summary of Analysis of Variance of Paradoxical Sleep Epoch Length

Source	SS	df	MS	F	p
Photoperiod	0.057	2	0.029	0.337	
Completeness	0.067	1	0.067	0.779	
Photoperiod x Completeness	0.032	2	0.016	0.186	
Within Cell	1.033	12	0.086		

BIOGRAPHICAL SKETCH

Michael George Dube was born in Hartford, Connecticut, on February 6, 1949. He attended parochial and later public schools in Connecticut graduating with honors from Enfield High School in 1967. In September, 1967, he entered the Johns Hopkins University. During his junior and senior years he worked as a research assistant to Dr. Charles L. Goodrick in Physiological Psychology at NICHD. In May, 1971, he was awarded the Bachelor of Arts degree with a major in behavioral sciences. From September, 1971, until the present he has been a research assistant to Dr. Wilse B. Webb while pursuing his studies in physiological psychology and neuroscience at the University of Florida. He received the Master of Arts degree in June, 1973. Following receipt of the Ph.D., he plans to continue research in the area of behavioral chronobiology. Mr. Dube is a member of several scientific societies and the Vermont Historical Society.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Wilse B. Webb, Chairman
Graduate Research Professor of Psychology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

John B. Munson
Associate Professor of Neuroscience

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Carol Van Hartesveldt
Associate Professor of Psychology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Donald A. Dewsbury
Professor of Psychology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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This dissertation was submitted to the Graduate Faculty of the Department of Psychology in the College of Liberal Arts and Sciences and to the Graduate Council, and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

March 1980

Dean, Graduate School